

Appl. No. 10/826,919
Amdt. Dated March 3, 2010
Reply to Office action of September 3, 2009

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1 – 51 (Cancelled).

52 (Original). A method of producing in a eukaryotic cell at least one protein comprising at least one unnatural amino acid, the method comprising:

growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises an unnatural amino acid and the eukaryotic cell comprises:

an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon;
and,

an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS comprises an amino acid sequence that corresponds to SEQ ID NO.: 48 – 53.

53 (Currently Amended). A method of producing in a eukaryotic cell at least one protein comprising at least one unnatural amino acid and modifying said protein, the method comprising:

growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises the unnatural amino acid and the eukaryotic cell comprises an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon and an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS is selected from:

(i) a *p*-propargyloxyphenylalanine O-RS that preferentially aminoacylates the O-tRNA with *p*-propargyloxyphenylalanine, and

(ii) a *p*-azido-L-phenylalanine O-RS that preferentially aminoacylates the O-tRNA with *p*-azido-L-phenylalanine, the *p*-azido-L-phenylalanine O-RS comprising :

(i) an amino acid sequence set forth in any one of SEQ ID NOs: 48 – 53[[.]] and conservative variants thereof, ~~which wherein the~~ conservative variants are at least 90% 98% identical to SEQ ID NOs 48 – 53, or

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(ii) an amino acid sequence that is a conservative variant of SEQ ID NO: 2, which conservative variant is at least 98% identical to SEQ ID NO: 2 that of a naturally occurring tyrosyl-aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of *E. coli*-TyrRS; aspartate at a position corresponding to Asn126 of *E. coli*-TyrRS; asparagine at a position corresponding to Asp182 of *E. coli*-TyrRS; alanine, or valine, at a position corresponding to Phe183 of *E. coli*-TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of *E. coli*-TyrRS;

incorporating into the protein the unnatural amino acid in the eukaryotic cell, wherein the unnatural amino acid comprises a first reactive group; and,

contacting the protein with a molecule that comprises a second reactive group; wherein the first reactive group reacts with the second reactive group to attach the molecule to the unnatural amino acid through a [3+2] cycloaddition, thereby modifying the protein.

54 (Original). The method of claim 53, wherein the molecule is a dye, a polymer, a derivative of polyethylene glycol, a photocrosslinker, a cytotoxic compound, an affinity label, a derivative of biotin, a resin, a second protein or polypeptide, a metal chelator, a cofactor, a fatty acid, a carbohydrate, or a polynucleotide.

55 (Cancelled).

56 (Previously Presented). The method of claim 53, wherein the O-RS is the *p*-propargyloxyphenylalanine O-RS, the unnatural amino acid is *p*-propargyloxyphenylalanine, the first reactive group is an alkynyl moiety and the second reactive group is an azido moiety.

57 (Previously Presented). The method of claim 53, wherein the unnatural amino acid comprises a *p*-propargyloxyphenylalanine.

58 (Currently Amended). The method of claim 53, wherein the O-RS is the *p*-azido-*L*-phenylalanine O-RS, the unnatural amino acid is *p*-azido-*L*-phenylalanine, the first reactive group is an azido moiety, and the second reactive group is an alkynyl moiety.

59 (Previously Presented). The method of claim 53, wherein the unnatural amino acid comprises a *p*-azido-*L*-phenylalanine.

60 – 61 (Cancelled).

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62 (Withdrawn). The method of claim 53, wherein the O-RS is the *p*-propargyloxyphenylalanine O-RS which *p*-propargyloxyphenylalanine O-RS comprises an amino acid sequence set forth in any one of SEQ ID NOS: 54-63, and conservative variants thereof, which conservative variants are at least 90% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprise two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of *E. coli* TyrRS; aspartate at a position corresponding to Asn126 of *E. coli* TyrRS; asparagine at a position corresponding to Asp182 of *E. coli* TyrRS; alanine, or valine, at a position corresponding to Phe183 of *E. coli* TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of *E. coli* TyrRS.

63 (Currently Amended). A method of producing in a yeast cell at least one protein of interest comprising at least one unnatural amino acid selected from *p*-azido-L-phenylalanine and *p*-propargyloxyphenylalanine, the method comprising growing, in an appropriate medium, the yeast cell, wherein the medium comprises the unnatural amino acid, and wherein the yeast cell comprises:

(a) a nucleic acid encoding said protein of interest and comprising at least one selector codon; and;

(b) an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon, wherein the O-tRNA is derived from an *Escherichia coli* tRNA; and,

(c) an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS is derived from an *Escherichia coli* aminoacyl tRNA synthetase.

64 (Currently Amended). A method of producing in a eukaryotic cell at least one protein comprising at least one unnatural amino acid selected from *p*-azido-L-phenylalanine and *p*-propargyloxyphenylalanine, the method comprising:

(a) growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises the unnatural amino acid and wherein the eukaryotic cell comprises an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon and an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS is selected from:

(i) a *p*-propargyloxyphenylalanine O-RS that preferentially aminoacylates the O-tRNA with *p*-propargyloxyphenylalanine, or

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(ii) a *p*-azido-L-phenylalanine O-RS that preferentially aminoacylates the O-tRNA with *p*-azido-L-phenylalanine, the *p*-azido-L-phenylalanine O-RS comprising:

(i) an amino acid sequence set forth in any one of SEQ ID NOs: 48 – 53[[L]] and conservative variants thereof, which wherein the conservative variants are at least 90% 98% identical to SEQ ID NOs 48 – 53, or

(ii) an amino acid sequence that is a conservative variant of SEQ ID NO: 2, which conservative variant is at least 98% identical to SEQ ID NO: 2 that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of *E. coli* TyrRS; aspartate at a position corresponding to Asn126 of *E. coli* TyrRS; asparagine at a position corresponding to Asp182 of *E. coli* TyrRS; alanine, or valine, at a position corresponding to Phe183 of *E. coli* TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of *E. coli* TyrRS; and,

(b) incorporating into the protein the unnatural amino acid in the eukaryotic cell.